REMARKS/ARUGMENTS

Upon entry of this amendment, claims 1-12 will remain canceled without prejudice or disclaimer, claims 15, 17-19 and 24 will be amended, whereby claims 13-25 will be pending. Claims 13, 15, 17-19, 20, 22 and 24 are independent claims.

The claims have been amended herein to even more clearly recite Applicants' invention by making what should be considered to be cosmetic changes to the claims. Therefore, the amendment should not be considered to raise new issues, and should be properly enterable after final rejection. In particular, the amendments to the claims should not raise new issues that would require further search or consideration, nor do the amendments raise a new matter issue.

Reconsideration and allowance of the application are respectfully requested.

Submission Of Supplemental Information Disclosure Statement

Applicants express appreciation for the inclusion with the Final Office Action of an initialed copy of the Form PTO-1449 submitted with the Supplemental Information Disclosure Statement filed September 10, 2003, whereby the Examiner's consideration of the Supplemental Information Disclosure Statement is of record.

Response To Prior Art Rejections

The following rejections are set forth in the Official Action:

(a) Claims 13-25 are rejected under 35 U.S.C. 102(b) as being anticipated by Arteel et al. (hereinafter "Arteel"), Chem. Res. Toxic., Vol. 12, 1999, pages 264-269. In this ground of rejection, the Examiner asserts that Arteel teaches every element of the claims, and refers to the

abstract for mammalian disclosure, and apparently page 264, left column, second paragraph, lines 1-3, and right column, first paragraphs lines 7 and 8.

(b) Claims 13-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combined teachings of Arteel and Muller et al. (Biochemical Pharmacology, 1984). In this ground of rejection, the Examiner asserts that Arteel inherently discloses Applicants' invention, and, if not, Applicants' invention would be obvious because Muller teaches that ebselen is an enhancer of peroxidase activity. In particular, the rejection asserts that Arteel teaches that ebselen is a substrate being reduced by NAPDH and thioredoxin reductase.

With respect to the rejections of record, Applicants note that Arteel is directed to a study involving materials that are similar to those used in Applicants' processes. Therefore, the rejections appear to be asserting that because similar materials are included in Arteel's study, the function of the materials in Arteel's study would be the same as recited in Applicants' claims. However, in contrast to the assertions in the rejections, Arteel taken alone or modified with Muller does not teach or suggest Applicants' disclosed and claimed invention.

In contrast to the assertions in the rejections, Arteel does not teach or suggest the use of ebselen as a substrate for thioredoxin reductase. Arteel is performing experiments with respect to the activity of mammalian thioredoxin reductase as a peroxynitrite reductase. In performing the study, Arteel infuses peroxynitrite to maintain a 0.2 μM steady-state concentration in potassium phosphate buffer. Arteel uses benzoate hydroxylation and nitrite formation as indices of oxidation reactions of peroxynitrite and of peroxynitrite reduction. Arteel particularly notes that when selenocystine or ebselen are present in the reaction mixture, there is a significant suppression of benzoate hydroxylation and an increase in nitrite formation until the NAPDH was

oxidized. **Arteel particularly specifies that the addition of thioredoxin did not enhance these effects** (page 264, in the Abstract).

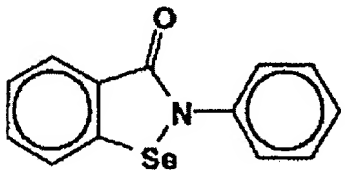
Therefore, Arteel is nonenabling for processes wherein thioredoxin is present as Arteel does not teach or suggest any need for having the thioredoxin present in the reaction.

Still further, Arteel discusses the formation of ebselen selenoxide by oxidation of ebselen by bolus addition of peroxynitrite. For example, the Examiner's attention is directed to Arteel, page 265 under the heading "Formation of Ebselen Selenoxide and Reduction by TR" and page 267 under the heading "Reduction of Ebselen Selenoxide by TR".

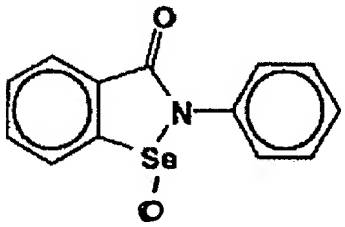
Thus, Applicants note that it is apparent that Arteel is directed to the investigation of the activity of mammalian thioredoxin reductase as a peroxynitrite reductase. Applicants do not that Arteel performs experiments with ebselen, such as disclosed at page 265, right-hand column. Moreover, Arteel discusses, at page 268, the right-hand column, an affinity of ebselen for thioredoxin reductase, and the role of thioredoxin in the thioredoxin reductase-albumin complex. However, Applicants once again direct the Examiner's attention to Engman et al., "Diaryl chalcogenides as selective inhibitors of thioredoxin reductase and potential antitumor agents", Anticancer Res. 1997 Nov-Dec;17(6D):4599-605. From a review of this document, it can be seen that Engman as well as **Arteel do not disclose the use of ebselen as a substrate for thioredoxin reductase**. Instead, Arteel pertains to ebselen selenoxide created by incubation with peroxynitrite. In its results and discussion, at page 268, right column, at the top of the column, Arteel cites Engman (Reference No. 22) for its disclosure of ebselen being an inhibitor of thioredoxin reductase, and discusses a mechanism that is not in conformance with that of the presently disclosed and claimed invention. Furthermore, Arteel does not teach or suggest any

effect of thioredoxin reductase with ebselen. Therefore, this prior art, as stated in Engman merely discloses, "The organoselenium compound Ebselen was found to be a competitive inhibitor of human thioredoxin reductase (Ki 2.8 microM), while a number of organotellurium compounds were found to be noncompetitive inhibitors (Kis 2.3 to 35.2 microM)." The prior art at most teaches that ebselen is an inhibitor of thioredoxin reductase, and that ebselen selenoxide can be a substrate. **However, Applicants' claims do not include ebselen selenoxide.**

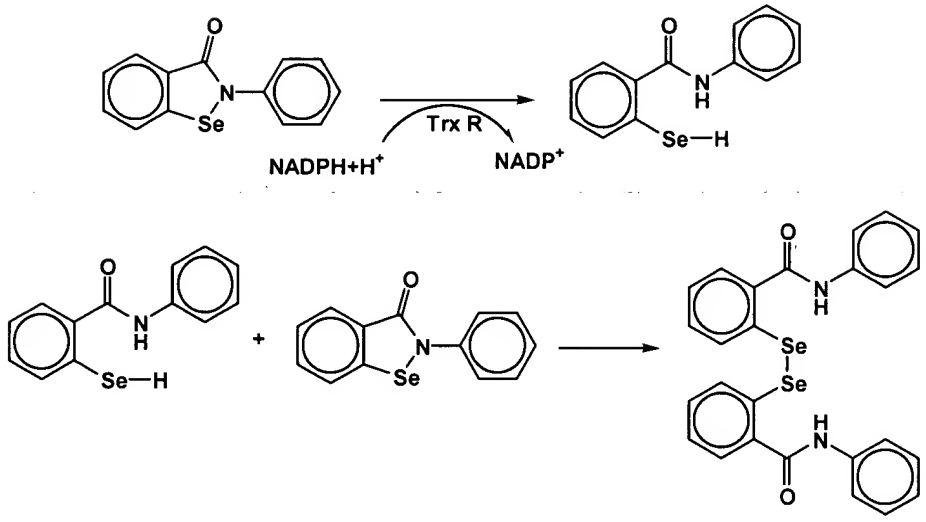
To assist the Examiner's further understanding of Applicants' invention, Applicants note that ebselen has the following formula:



Ebselen selenoxide has the following formula:



Moreover, the reaction of ebselen in Applicants' system does not form ebselen selenoxide, the compound disclosed in Arteel, but produces compounds according to the following reaction scheme, as disclosed on pages 13 and 14 of Applicants' specification.



Therefore, summarizing the above, Arteel discloses that:

(1) Ebselen inhibits thioredoxin reductase, and it is therefore expected that addition of ebselen would shut off effects of thioredoxin reductase.

(2) The strong oxidant peroxynitrite is reduced by thioredoxin reductase provided that ebselen is present. The mechanism being that ebselen is oxidized to ebselen selenoxide which is reduced by thioredoxin reductase.

(3) There is no effect by adding thioredoxin.

In contrast, according to Applicants' invention and included in Applicants' claims, the substrate, such as ebselen, is a substrate for the thioredoxin reductase, not an inhibitor. In Applicants' invention, there is a fast reaction of the thioredoxin reductase directly with ebselen to the selenol which is reoxidized removing peroxides. Ebselen provides a fast reaction of reduced thioredoxin. That is ebselen itself is also a directed substrate for thioredoxin . The addition of ebselen will therefore oxidize thioredoxin which explains its anti-inflammatory effects. **Thioredoxin oxidation as recited in Applicants' claims is not taught or suggested in any prior art of record.**

Applicants once again respectfully submit that a substance which is an inhibitor of an enzyme is not a substrate unless it is a suicide substrate, which kills the enzyme by covalent modification. For example, the Abstract in Arteel merely states in the last sentence that, "In parallel experiments, thioredoxin reductase efficiently reduced ebselen selenoxide back to ebselen". Moreover, in the above-noted portion of page 268 of Arteel, it is stated that, "Ebselen has been shown previously to have an affinity for TR, competitively inhibiting the Trx-dependent reduction of insulin by TR with an apparent K_i of 2.8 μM (22)." Arteel shows no effect of thioredoxin on reduction of ebselen selenoxide by NADPH and thioredoxin reductase.

Regarding the use of Muller, the Examiner is reminded that Applicants' specification discloses at page 2, lines 2-6, that Muller discloses that Applicants' compounds can reduce a peroxide (active oxygen) by glutathione peroxidase-like activity. However, the specification points out that the reduction of a peroxide by glutathione peroxidase is based on a totally different mechanism from that proceeded by thioredoxin reductase.

In particular, Muller is directed to the glutathione peroxidase-like activity of ebselen in vitro, in contrast to its sulfur analog, PZ25, and to its antioxidant activity. Muller discloses glutathione peroxidase and glutathione, and does not teach or suggest any relation of this activity to thioredoxin reductase activity, thioredoxin activity and/or thioredoxin/thioredoxin reductase activity. Also, glutathione is a small peptide acting non-enzymatically, and therefore requires high concentrations. It is a different mechanism than the enzymatic system of the present invention.

Thus, Applicants respectfully submit that the prior art of record does not provide any motivation for using the disclosure of Muller to explain Arteel.

The prior art does not teach or suggest the invention as disclosed and claimed by Applicants. Thus, amongst other deficiencies in the prior art of record, the prior art does not teach or suggest that ebselen is an outstanding substrate for reduced thioredoxin. This is not taught or suggested in the prior art of record and, without wishing to be bound by theory, is an important manner in which ebselen hinders inflammation by preventing thioredoxin from reducing and activating a range of transcription factors, including NFkB. **Thus, ebselen can target both thioredoxin reductase and thioredoxin with separate results.**

Moreover, as noted above, prior to Applicants' invention, one having ordinary skill in the art would be under the belief that ebselen is an inhibitor of thioredoxin reductase, and not a substrate as disclosed and claimed by Applicants.

In contrast to the prior art of record, the present invention demonstrates that ebselen is a substrate being reduced by NADPH and thioredoxin reductase with a low Km-value meaning that it is a very good substrate undergoing unlimited cycles of oxidation reduction in the presence of hydrogen peroxide without affecting the activity of the enzyme. The reduced ebselen is called ebselen selenol and has the Se-N bond broken by reduction. The selenol is oxidized back to ebselen by hydrogen peroxide or another peroxide and a new cycle starts. The reaction is ultimately driven by NADPH. Reduced thioredoxin strongly enhances the thioredoxin reductase reaction which is also proven by determination of the rate of reduction of ebselen by reduced thioredoxin using kinetics with tryptophan fluorescence. The result, never seen before, is that ebselen is a very efficient oxidant of reduced thioredoxin.

Applicants note that the Examiner's comments in the Final Office Action acknowledge Applicants' arguments, but assert that a concentration has not been claimed. In contrast to this

assertion, Applicants were not arguing concentrations, but were referring to reaction rates, and the unexpected fact reaction associated with Applicants' methods.

Being more specific to the pending claims, Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 13, a method for reduction of a substrate with thioredoxin reductase, comprising combining the thioredoxin reductase, the substrate and NADPH under conditions to reduce the substrate, the substrate comprising a substance selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof. For example, the prior are utilized in the rejection does not teach or suggest Applicants' method let alone such a method include substrates as disclosed and claimed by Applicants. As noted above, Arteel discloses ebselen oxide in the reaction which is not included in Applicants substrates.

Still further, Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 15, a method of enhancing peroxidase activity of thioredoxin reductase, comprising combining NAPDH, thioredoxin reductase, thioredoxin and a substrate under conditions to enhance peroxidase activity of thioredoxin reductase, the substrate comprising a substance selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof.

Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 17, a method of oxidizing reduced thioredoxin by a substrate, the method comprising combining reduced thioredoxin and a substrate

under conditions to oxidize the reduced thioredoxin with the substrate, the substrate comprising a substance selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof.

Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 18, a method for reducing a peroxide comprising combining thioredoxin, thioredoxin reductase, NAPDH and a substrate under conditions to reduce the peroxide, the substrate comprising a substance selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof.

Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 19, a method of preventing peroxidation of a substance comprising combining thioredoxin, thioredoxin reductase and NADPH with a substrate under conditions to prevent peroxidation of the substance, the substrate being selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof.

Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 20, a method for enhancing peroxidase activity of thioredoxin reductase in vivo which comprises administering a peroxidase activity enhancing effective amount of a substrate to a mammal, the substrate comprising a substance selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof.

Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 18, a method of reducing a peroxide in vivo which comprises administering an peroxide reducing effective amount of a substrate to a mammal, the substrate comprising a substance selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof.

Applicants respectfully submit that Arteel, whether taken alone or in view of Muller, does not teach or suggest, as recited in Applicants' independent claim 24, a method of preventing peroxidation of a substance in vivo by oxidizing reduced thioredoxin in a peroxidase reaction proceeded by thioredoxin reductase comprising administering a peroxidation preventing effective amount of a substrate to a mammal, the substrate being selected from the group consisting of a compound represented by the following general formula (1) or (1') and a physiologically acceptable salt thereof, and a hydrate thereof and a solvate thereof.

Still further, Applicants' dependent claims further define Applicants' invention, and are patentability for the combination of features recited therein.

For the reasons set forth above, the methods recited in Applicants' claims are not taught or suggested by the prior art, whereby the claims are patentable over the prior art of record, and the rejections should be withdrawn.

CONCLUSION

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejections of record, and allow each of the pending claims.

Applicants therefore respectfully request that an early indication of allowance of the application be indicated by the mailing of the Notices of Allowance and Allowability.

Should the Examiner have any questions regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,
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